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Busso, Juan Pablo ; Blanckenhorn, Wolf U

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Original Article

Disruptive sexual selection on male body size in the polyphenic black scavenger fly *Sepsis thoracica*

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Sexual selection has 2 main components, female preference and male–male competition, which can lead males to adopt alternative reproductive tactics to optimize their reproductive success. Two traits that significantly influence reproductive success are body size and coloration, as they can facilitate access to females through male contests or as female attractors. We investigated whether, and if so which mechanism of sexual selection contributes to the maintenance, and possibly even the establishment, of 2 almost discrete male morphs in the polyphenic black scavenger fly *Sepsis thoracica* (Diptera: Sepsidae): small and black, or large and amber. We performed 2 complementary laboratory experiments to evaluate the mating success of the different male morphs and the behaviors (of both males and females) presumably mediating their mating success. We found evidence for intraspecific disruptive sexual selection on male body size that is mediated by male–male interactions, and significant positive directional selection on body size that interacted with (directional) selection on coloration, likely contributing to the origin and/or maintenance of the threshold relationship between the 2 traits in this species. The simultaneous occurrence of disruptive selection and polyphenism in *S. thoracica* supports the role of sexual selection in the intraspecific diversification of coupled traits (here body size and coloration), which could be a speciation starting point.

Key words: body size, Diptera, female preference, fly, male–male competition, mating, melanism, polymorphism, threshold trait, trade-off.

INTRODUCTION

Intraspecific phenotypic diversity can be significantly influenced by sexual selection (Andersson 1994; Gray and McKinnon 2007). In some animals, sexual selection not only mediates the evolution of sexual dimorphism (Fairbairn et al. 2007), but also dimorphism within one sex (Rios-Cardenas et al. 2007; Hurtado-Gonzales et al. 2010). In many cases, such polymorphisms (based on genetic differences) or polyphenisms (induced by the environment) are maintained by divergent selection across populations; at least in theory such disruptive sexual selection can promote sympatric speciation, a mode of speciation under controversy (van Doorn et al. 2004; Gavrilets and Hayashi 2005; Gray and McKinnon 2007). However, this macro-evolutionary process is ultimately grounded in micro-evolutionary disruptive selection within populations, for which there is comparatively little support (Gray and McKinnon 2007).

One of the main components of sexual selection is male–male competition (reviewed by Andersson 1994; Hunt et al. 2009).

Strong competition for mates can lead males to adopt different strategies to obtain mates, resulting in alternative reproductive tactics (Oliveira et al. 2008). Gaining privileged access to mates bears costs; as resources are limited, individuals should tailor their investment into different traits so as to optimize their reproductive success through either alternative tactic. When these differential investments are mutually exclusive, as is the case for many trade-offs, they can result in intrasexual polyphenisms (Taborsky and Brockmann 2010). Besides physiological allocation costs, trade-offs can impose more general costs in terms of overall fitness whenever a beneficial change in one trait results in a detrimental change in another (Stearns 1989; Leroi et al. 1994). In such situations polyphenisms may continue to be favored by disruptive selection, reducing the fitness of intermediate phenotypes (Brockmann et al. 2008; Danforth and Desjardins 1999).

The other main component of sexual selection is mate choice, most commonly exerted by females (Andersson and Simmons 2006; Hunt et al. 2009). Females are regularly choosier because they face higher reproductive costs than males (Bateman 1948; Clutton-Brock 1988). A strong female preference can cause divergence in male phenotypes, which can drive males to adopt alternative

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reproductive tactics, either to invest in costly structures for mate attraction (bourgeois tactic), or omit these costs and exploit the investment of their bourgeois conspecifics (parasitic tactic; Oliveira et al. 2008). Females typically prefer bourgeois males, thereby exerting intersexual selection on them, while the parasitic males circumvent such preferences (Gross 1991; Shuster and Sassaman 1997; Watson and Simmons 2010). Therefore, female preferences may result in disruptive sexual selection whenever males differentially invest in such mutually exclusive functions (Taborsky and Brockmann 2010). Moreover, female choice can be variable, for instance, when females prefer different traits in different males, which can also result in disruptive sexual selection (Sappington and Taylor 1990; Greene et al. 2000; Stelkens et al. 2008; Busso and Davis Rabosky 2016).

Male–male competition and female preference are not mutually exclusive, so they can act simultaneously in a species (Hunt et al. 2009). Body size is one of the most important traits often favored by classic male–male competition and/or female choice (reviewed by Andersson 1994; Blanckenhorn 2000; Fairbairn et al. 2007; Hunt et al. 2009). Larger body sizes normally facilitate access to females either through victory in aggressive contests between males or by forcing copulations (Partridge and Farquhar 1983; Anderson and Fedak 1985; Clutton-Brock 1988; Zucker and Murray 1996; Shine and Mason 2005). Larger body sizes are also often favored by female preferences (Simmons 1992; Brown et al. 1996; MacLaren and Rowland 2006). These benefits of body size in mate acquisition are particularly evident in species that display alternative reproductive tactics (Dominey 1980; Emlen 1997; Taborsky and Brockmann 2010). In such species, discrimination against intermediate body sizes can result in disruptive selection on male body size (Danforth and Desjardins 1999).

Another trait that also plays an important role in sexual selection is coloration (Andersson 1994; Lozano 1994). Coloration can influence male–male competition when it functions as a signal to other males (Pärt and Qvarnström 1997; Pérez I de Lanuza et al. 2013). It can also influence female preference when it functions to attract females (Kodric-Brown 1985; Lozano 1994), or it can be a simultaneous signal for both sexes (Kodric-Brown 1996; Pérez I de Lanuza et al. 2013). Through either of these mechanisms, therefore, disruptive selection on coloration may result in polyphenisms (Sappington and Taylor 1990; Greene et al. 2000).

Studies focusing on polymorphic species typically address the mechanisms that can (stably) maintain morphs in nature, such as negative frequency-dependent selection (Maynard Smith 1982; Kokko et al. 2007; Zajitschek and Brooks 2008). We here address the role of disruptive sexual selection as a force maintaining or even widening the intraspecific phenotypic differences between morphs. We focus on the black scavenger fly *Sepsis thoracica* (Diptera: Sepsidae), which presents 2 male morphotypes: a small, black (obsidian) and a large, yellow (amber) morph, while females are always totally black (Busso et al. 2017). These differences are largely environmentally induced, hence a polyphenism, as body size, coloration, and fly behavior are only weakly genetically differentiated across Europe (Busso and Blanckenhorn 2018a). In other sepsid species, mating success is strongly influenced by individual body size and can involve both female preference and male–male competition (Ward 1983; Zerbe 1993; Blanckenhorn et al. 2000; Eberhard 2002; Puniamoorthy, Blanckenhorn, et al. 2012; Puniamoorthy, Schäfer, et al. 2012). By analyzing male and female behaviors during mating encounters, we here investigated whether, and if so by which mechanism sexual selection contributes to the maintenance

and possibly even the establishment of the 2 male morphs in *S. thoracica*. If male–male competition is more important, we expected significant behavioral differences between the morphs, whereas if female preferences play a greater role, we expected female rejection responses to differ between male morphs. We further asked if the male polyphenism of *S. thoracica* represents alternative reproductive tactics. This work should contribute to our ultimate goal, to understand the evolution of the male polyphenism in this species.

MATERIALS AND METHODS

Sepsis thoracica maintenance and rearing

We sampled 7 European *S. thoracica* populations from Pehka, Estonia (59.48° N, 26.37° W), Ludwigshafen, Germany (49.48° N, 8.42° W), Asturias, Spain (43.3° N, 6.0° E), Petroia, Italy (43.23° N, 12.56° W), Padula, Italy (40.34° N, 15.66° W), Lagonero, Italy (40.14° N, 15.75° W), and Lamezzia, Italy (38.92° N, 16.25° W). Wild-caught females were brought to the laboratory and used to establish multiple (5–25) replicate iso-female line cultures per population that were housed in separate plastic containers at 18 ± 1 °C under a 14:10 h light:dark cycle. The cultures were regularly supplied with fresh cow dung, sugar, and water ad libitum.

To generate flies for our sexual selection experiments, containers with dung were placed for 24 h inside any of the *S. thoracica* stock cultures for females to deposit eggs. This dung was incubated afterwards in another container until experimental adult flies emerged. To guarantee virginity, females and males were separated upon emergence under a stereo microscope MZ12 (Leica). All flies used in our experiments were 3 to 7 days old.

Estimation of sexual selection in the laboratory

For each experimental trial, we placed 4 amber and 4 obsidian males into a transparent plastic arena ($10 \times 10 \times 20$ cm³) and then introduced 4 females. This experiment using large groups of 12 unmarked individuals served to best emulate the natural situation at a dung pat. All flies of a given group stemmed from the same isofemale line from a given location, and over time a total of $N = 90$ trials were performed, in random order, with flies from many different lines and locations to generally represent the species. Females were picked randomly, and males were chosen by eye as being either black or fully amber, which was later verified by the measuring procedure described below, leading to occasional intermediate phenotypes (blue in Figure 1) that were excluded from analyses post-hoc. Based on preliminary trials, all behavioral interactions were observed until all females were paired or otherwise stopped after 20 min.

We scored, as proxies for male–male competition, the number of fights and wavings between individual males. We noted the morph of the performer and receiver of these actions (without knowing the individual's identity), as well as the number of individuals of each morph present at the time of each action. A fight was defined as a male jumping on another male and/or knocking him over to the ground; waving consisted of simultaneous wing and abdomen movements directed to another individual, which we presumed (but not know for certain) to have aggressive display or signaling functions. We also measured the mounting latency (time elapsed until the male mounted a female to initiate copulation without getting off again), the number of mounting attempts until copulation took place or the trial ended, copulation latency (time elapsed until copulation started), and copulation duration (if it occurred; always

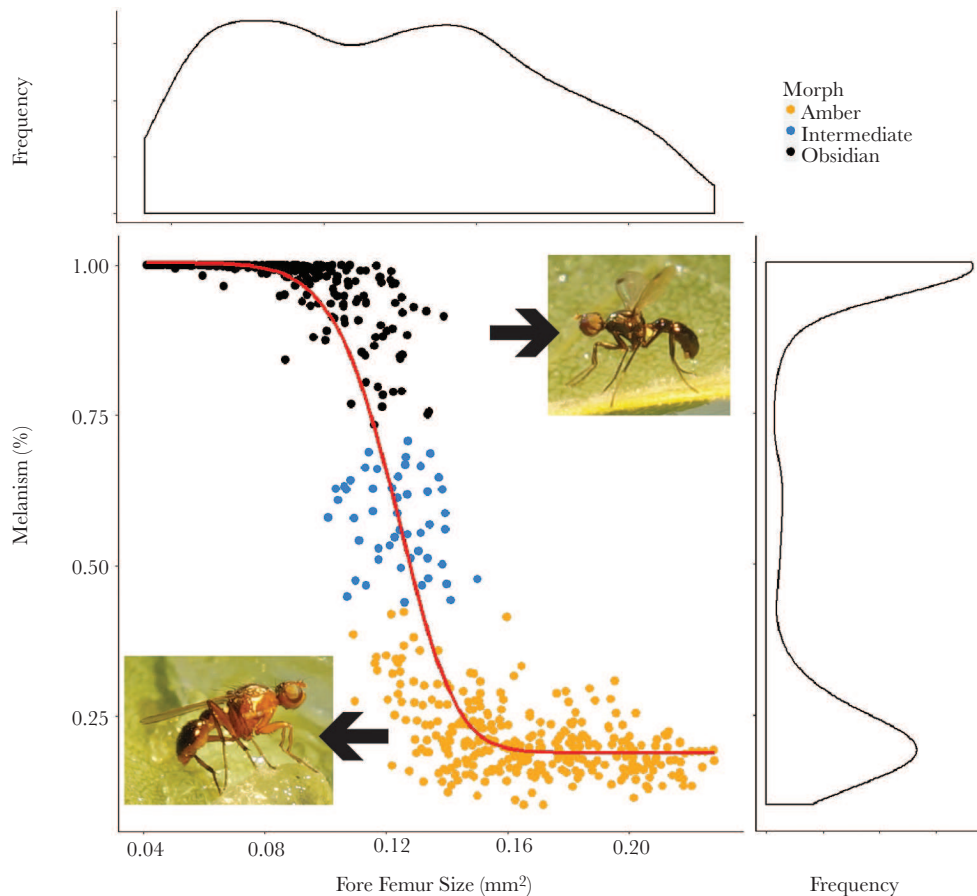


Figure 1

Relationship between body size and melanization of male *Sepsis thoracica* with their respective frequency distributions (data of the sexual selection experiment only). The pictures show the amber and obsidian male morphs.

measured fully, even beyond the 20-min threshold, as it lasts ca. 20 min on average).

As a proxy for female preference we measured female reluctance (time elapsed since the male mounted the female until she bent her abdomen upward allowing him to contact her genitalia). In other sepsids, one can measure female shaking as a proxy for female preference (e.g., Blanckenhorn et al. 2000), but we only observed few shaking events in our 90 trials, hence this variable was not further considered. We registered which male copulated with which female by isolating the mating pairs from the group, as individuals were unmarked.

After the experiment finished, we measured the body size of all mated and nonmated individuals. We scored the foreleg femur area as an excellent proxy for both body size and coloration, based on previous analyses (size and coloration of all body parts are highly correlated; Busso and Blanckenhorn 2018a). All flies were dissected to photograph their foreleg under a stereo microscope MZ12 with a DFC490 camera (Leica) against a neutral white background. The camera was calibrated with a mini IT-8 calibration target to guarantee color consistency between the different pictures. We used a self-written code in ImageJ that measured the number of pixels of the foreleg femur, and converted them to the real area according to the scale in each picture. The code also measured the number of melanic pixels in the femur as an objective quantification of melanism, i.e., coloration, varying from amber to black. Any pixel with a V-value over 163 in

the YUV color space was defined as melanic, corresponding to the valley in the bimodal V-value distribution of femur coloration (Y: brightness; U: blue-luminance; V: red-luminance; see <https://en.wikipedia.org/wiki/YUV>; cf. Figure 1, right; Busso and Blanckenhorn 2018a). Melanism represented the proportion of black and brown pixels over all the pixels of the femur (thus requiring a logistic approach).

Alternative reproductive tactics experiment

We performed a second experiment to quantify behavioral differences between the male morphs in more detail ($N = 174$). Before each trial, males were marked with a small dot of paint on the back of their thorax to distinguish individuals of the same morph. In this experiment, for each trial we placed 2 males (2 ambers, 2 obsidians, or one of each) in a transparent plastic arena ($10 \times 10 \times 20 \text{ cm}^3$) and then introduced 1 female. The order of male introduction was random. During the trials, we scored the number of fights and wavings between any 2 individuals (as above), scoring the sex and morph of the performer and receiver of these actions. We additionally registered mounting attempts, mounting latency, copulation latency, and copulation duration, as above. In this experiment, we did not measure the body size of the individuals, just their size category defined by their color. Interactions were observed for 20 min or until the female was paired with one or the other male.

Statistical analyses of the sexual selection experiment

We used standard regression methods to estimate uni- and bivariate linear and quadratic (nonlinear) selection coefficients (Lande and Arnold 1983; Arnold and Wade 1984) assessing the intensity of sexual selection on fore femur (i.e., body) size and melanism in *S. thoracica* males in the sexual selection experiment. Univariate coefficients integrate direct and indirect selection on the traits, while bivariate coefficients indicate the selection on one trait controlling for selection on another. This permits disentangling selection on multiple traits even when correlated, and also estimates the resulting direction of selection acting on each trait (Lande and Arnold 1983; Arnold and Wade 1984). To allow direct comparison, we converted absolute to relative mating success (z -scores). We calculated, separately for each trial, standardized z -scores for fore femur size and melanism by subtracting the trial mean from each value and dividing the difference by the standard deviation: $z_i = (x_i - \bar{x}) / SD_x$. Relative fitness in each trial was calculated as the dichotomous absolute fitness (i.e., mated or not [1 or 0]) divided by the trial mean fitness, i.e. the proportion of mated males (Arnold and Wade 1984). We used models of relative fitness on z -scored body size and melanism, $w = c + \beta_1 z$, to estimate univariate linear selection coefficients for each variable, where w is the relative fitness, β_1 is the univariate linear selection coefficient, and z is the standardized independent variable (body size or melanism). c is the intercept in all models. To estimate bivariate linear selection, we used the model $w = c + \beta_{2bs} z_{bs} + \beta_{2m} z_m$, where β_{2bs} and β_{2m} are the bivariate linear selection coefficients of body size and melanism respectively, and z_{bs} and z_m refer to standardized body size and melanism. For the univariate quadratic (nonlinear) selection we used the model $w = c + \beta_1 z + \gamma_1 z^2$, where γ_1 is half the univariate quadratic selection coefficient; and for the bivariate quadratic selection differentials we used the model $w = c + \beta_{2bs} z_{bs} + \beta_{2m} z_m + \gamma_{2bs} z_{bs}^2 + \gamma_{2m} z_m^2 + \gamma_{2bs \times m} z_{bs} z_m$, where γ_{2bs} and γ_{2m} are half the bivariate quadratic selection coefficients of body size and melanism, respectively, and $\gamma_{2bs \times m}$ is the correlational (i.e., interactive) selection between the 2 traits. The linear terms of the bivariate models are not interpreted, as these equations with higher order terms serve to measure only how selection influences the variances and covariances of traits when linear selection effects are controlled (Lande and Arnold 1983). Significance of each selection coefficient in these multiple regressions was tested with the corresponding binomial models with binary (i.e., absolute) mating success (rather than relative fitness) as the outcome. We incorporated in all models a random effect of maternal line nested within populations to control for fly relatedness, and also the trials as random effect to control for differences between trial conditions.

Because unmarked males of the same morph could not be individually differentiated in the sexual selection experiment, we calculated fights and wavings per morph. We analyzed the fighting or waving differences between the morphs with a generalized mixed effect model, fitting a negative binomial to the data. The negative binomial fitted the data better than the Poisson model and solved the overdispersion problem. We included as factors in the model the morph of the performer, the morph of the receiver and the interaction between them ($N = 90$ trials). We also incorporated in the model the random effects of line nested within population and the trials (Briffa et al. 2013), as above. The model also contained the following offsets: duration of the trial, number of performers (number of individuals of each morph that could perform

an action), and number of receivers (number of individuals of each morph that could receive the action). These offsets controlled for the observation time and also for the individuals present when an action was recorded, hence the output is directly interpretable as rates per minute, performer, and receiver (Reitan and Nielsen 2016). Fights and wavings were analyzed similarly but separately.

For the mounting attempts, we fitted a Poisson model with male size, female size, morph, and all possible 2-way interactions between them. We also incorporated in the model line nested within populations and trials as random effects. The offset in this model was the mounting latency, or alternatively trial duration if there was no copulation. We took mounting latency whenever the male mounted the female and this lead to copulation, in which case the mated pair did not interact anymore with the other males. There were only 2 cases where another male took over a female, so these trials were excluded from the analyses. For mounting latency, copulation latency and female reluctance ($N = 248$ matings), we fitted a generalized mixed effect model with a negative exponential distribution, and for copulation duration ($N = 248$) a linear mixed effect model with underlying normal distribution, always including the same explanatory variables and random effects as above but excluding any offsets.

To check for assortative mating, we fitted a linear mixed effect model where the outcome variable was relative male size (standardized per trial) and the predictor variables were relative female size (standardized per trial), male morph, and the interaction between the two ($N = 248$). We also incorporated in the model line nested within populations and trial as random effects.

Statistical analyses of the alternative reproductive tactics experiment

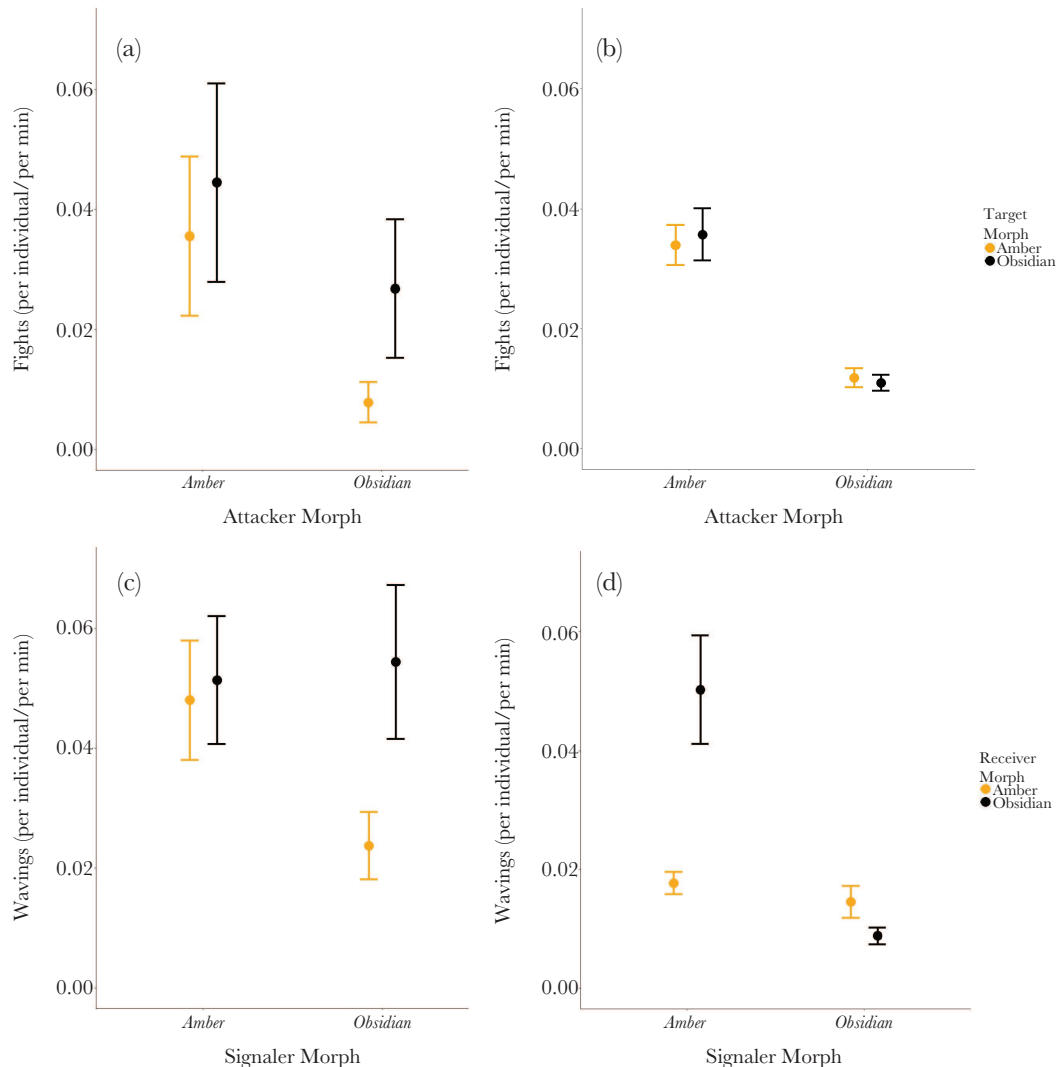
We analogously analyzed the alternative reproductive tactics experiment with a generalized mixed effect model, fitting a negative binomial regression to the fights and wavings (as above), including the factors morph of the performer, morph of the receiver, and the interaction between them ($N = 87$ trials). We here additionally included the color marking to detect if it influenced the fly's behavior. The models also contained the random effects of line nested within population and trial. The offset in this case was mounting latency or trial duration, as above. Mounting attempts, mounting and copulation latency, and copulation duration ($N = 24$ matings) were analyzed as in the sexual selection experiment.

We additionally analyzed the influence of the fighting and waving behaviors on individual copulation success with a generalized mixed effect model, fitting a logistic binomial regression to the data. The model included the predictor variables fighting, waving, morph, opponent's morph, marking, and the bivariate interactions fighting:morph, waving:morph, fighting:opponent's morph, and waving:opponent's morph. This model also contained the random effects line nested within population and trial. The offset in this case was again mounting latency or trial duration. All analyses were done using the software R Version 3.2.2 (R Development Core Team 2015).

RESULTS

Male-male interactions

The morphs had starkly different body sizes (Figure 1, $X^2 = 1106.38$, $P < 0.001$). In the sexual selection experiment amber males fought more than obsidians (Figure 2a, $X^2 = 6.90$,

**Figure 2**

Mean \pm 95% CI of fighting and waving frequencies in the sexual selection (a,c) and the alternative reproductive tactics experiments (b,d).

$P = 0.009$), and obsidians attacked more other obsidians than ambers (*fighter:receiver* $X_I^2 = 4.21$, $P = 0.040$; an effect however turning marginally nonsignificant when adjusting for simultaneous analysis of 2 traits), both morphs receiving roughly equal numbers of attacks (*receiver* $X_I^2 = 3.08$, $P = 0.080$). Ambers also waved more than obsidians (Figure 2c, $X_I^2 = 5.58$, $P = 0.018$), waving equally to both morphs, while obsidians waved mostly to other obsidians (*receiver* $X_I^2 = 28.73$, $P < 0.001$; *signaler:receiver* $X_I^2 = 20.19$, $P < 0.001$).

In the alternative reproductive tactics experiment, we also saw that ambers fight more than obsidians (Figure 2b, $X_I^2 = 6.12$, $P = 0.013$), but they were indifferent to the morph of the opponent (*receiver* $X_I^2 = 0.04$, $P = 0.846$; *fighter:receiver* $X_I^2 = 0.48$, $P = 0.488$). The marking did not significantly affect attack behavior ($X_I^2 = 2.39$, $P = 0.122$). For the wavings, results differed slightly from the sexual selection experiment, as ambers waved more than obsidians (Figure 2d, $X_I^2 = 11.38$, $P < 0.001$), and waved more to obsidians than to ambers, while the opposite was true for obsidians (*receiver* $X_I^2 = 0.83$, $P = 0.363$; *signaler:receiver* $X_I^2 = 6.40$, $P = 0.011$). Again, the marking did not significantly affect waving behavior ($X_I^2 = 0.01$, $P = 0.923$).

Of all variables and bivariate interactions originally included in the alternative reproductive tactics experiment model, fighting was the only trait that significantly affected the morphs' copulation success (Figure 3, $X_I^2 = 4.36$, $P = 0.037$); all other variables were not significant (wavings, $X_I^2 = 1.55$, $P = 0.213$; morph, $X_I^2 = 0.48$, $P = 0.489$; opponent's morph, $X_I^2 = 2.79$, $P = 0.095$; marking, $X_I^2 = 0.29$, $P = 0.591$; morph:opponent's morph, $X_I^2 = 0.08$, $P = 0.771$; fights:morph, $X_I^2 = 0.23$, $P = 0.631$; wavings:morph, $X_I^2 = 1.47$, $P = 0.226$; fights:opponent's morph, $X_I^2 = 1.38$, $P = 0.239$; wavings:opponent's morph, $X_I^2 = 0.49$, $P = 0.484$).

Male–female interactions

In the sexual selection experiment, we found no assortative mating, since standardized male size was not significantly correlated with standardized female size ($X_I^2 = 0.01$, $P = 0.942$), nor was there an interaction with morph ($X_I^2 = 1.37$, $P = 0.504$). The only significant effect was female body size positively influencing copulation duration, a well-known phenomenon in other sepsids (Puniamoorthy, Blanckenhorn, et al. 2012; $X_I^2 = 5.04$, $P = 0.021$). Since all other behavioral traits at least partly attributable to the female (reluctance and copulation latency) had no significant effects

in either experiment before or after necessary statistical Bonferroni correction (all model estimates listed in [Supplementary Tables S1 and S2](#)), we conclude that the contribution of female choice to sexual selection in these competitive laboratory settings is minor in this species, based on the variables considered.

Selection estimates (sexual selection experiment)

[Table 1](#) lists all univariate and bivariate linear and quadratic selection coefficients. To further illustrate the complex numerical interpretation of these coefficients, [Figure 4](#) depicts the fitness landscape of the males' morphospace resulting from the model including all parameters.

DISCUSSION

We presented evidence for ongoing disruptive sexual selection on body size, here primarily mediated by male–male competition ([Figures 2 and 3](#)), in *S. thoracica* (Diptera: Sepsidae; [Figure 4](#)), a dung fly species featuring a rare condition-dependent male dimorphism in size and coloration ([Figure 1](#)). Disruptive selection was accompanied by strongly positive directional selection on body size that interacted positively with selection on (orange) coloration (i.e., negatively with melanism), implying accelerating selection favoring large, amber males ([Table 1](#)). Nevertheless, small black males showed greater mating success than middle-sized and -colored males ([Figures 1 and 4](#)). The 2 male morphs differ quantitatively, but not qualitatively, in their mating behavior, however so far showing little evidence of hard-wired (i.e., genetic) alternative mating

tactics or strategies ([Figure 2](#)). We conclude that current sexual selection contributes to the maintenance, and in the past likely also contributed to the origin, of the 2 male morphs ([Danforth and Desjardins 1999](#); [Brockmann et al. 2008](#)).

Our evidence of disruptive sexual selection on male body size selecting against intermediate body sizes is based on the bivariate nonlinear (quadratic) selection coefficient $\gamma_{2_{bs}}$ for body size being positive ([Table 1](#), [Figure 4](#)), which concurs with the phenotypic subdivision of the males' morphospace ([Figure 1](#)). A strong influence of body size on mating success has also been demonstrated in related sepsid flies lacking male polyphenism (*S. cynipsea* and *S. punctum*: [Ward 1983](#); [Blanckenhorn et al. 2000](#); [Puniamoorthy, Blanckenhorn, et al. 2012](#); [Puniamoorthy, Schäfer, et al. 2012](#)), supporting the general idea that selection on size strongly affects the evolution of body size and sexual dimorphism of the entire clade ([Rohner et al. 2016](#)). Although most sepsids—a family of acalyptrate flies generally associated with decaying organic matter with ca. 300 species worldwide—are shiny-black, orange color recurs repeatedly throughout the clade ([Pont and Meier 2002](#); see <http://sepsidnet-rmbr.nus.edu.sg/> for pictures of many species). However, as far as we know *S. thoracica* is the only *Sepsis* species in Europe showing a male polyphenism in coloration strongly coupled with size ([Figure 1](#)). Strong selection on body size however was not accompanied by equally strong selection on melanism/coloration (one being the inverse of the other), which was relatively weaker overall ([Table 1](#)), despite the fact that bimodality is strong for coloration and weak to nonexistent for size ([Figure 1](#)). We had expected the opposite. Nevertheless, in line with the coupling of the 2 traits, correlational selection ($\gamma_{2_{bs \times m}}$) was strong, qualitatively reversing directional selection favoring melanism in the bivariate model (β_{2_m} ; albeit not significant) to significantly disfavor melanism, i.e., favor amber-colored (and large) males in the univariate model (β_{1_m} ; [Table 1](#)). Correlational selection at this point in time thus likely determines the precise position of the 2 fitness peaks in the trait space ([Figure 4](#)). This interactive (correlational) selection on

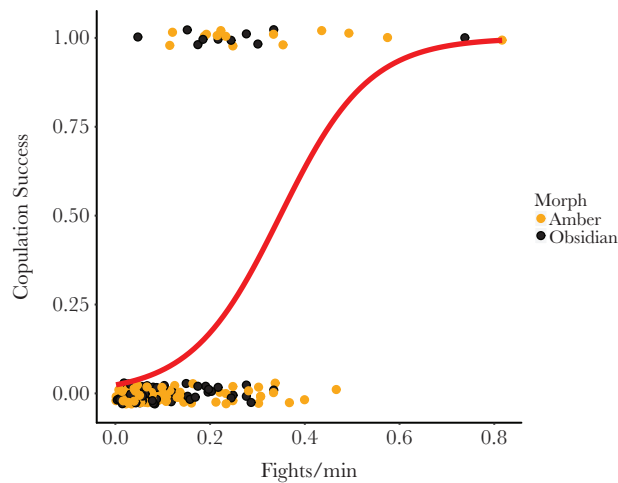


Figure 3
Sigmoid positive influence of fighting on mating success of both male morphs in the alternative reproductive tactics experiment.

Table 1
Selection coefficients (\pm 95% CI) for body size and melanism in the sexual selection experiment (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

Selection coefficient	Fore femur area (bs)	Melanism (m)
Univariate linear (β_1)	0.537 (± 0.144)***	−0.454 (± 0.146)**
Bivariate linear (β_2)	1.019 (± 0.442)*	0.510 (± 0.442)
Univariate quadratic (γ_1)	−0.052 (± 0.388)	−0.356 (± 0.634)
Bivariate quadratic (γ_2)	2.068 (± 1.900)*	0.764 (± 1.790)
Correlational ($\gamma_{2_{bs \times m}}$)	−1.791 (± 1.750)*	

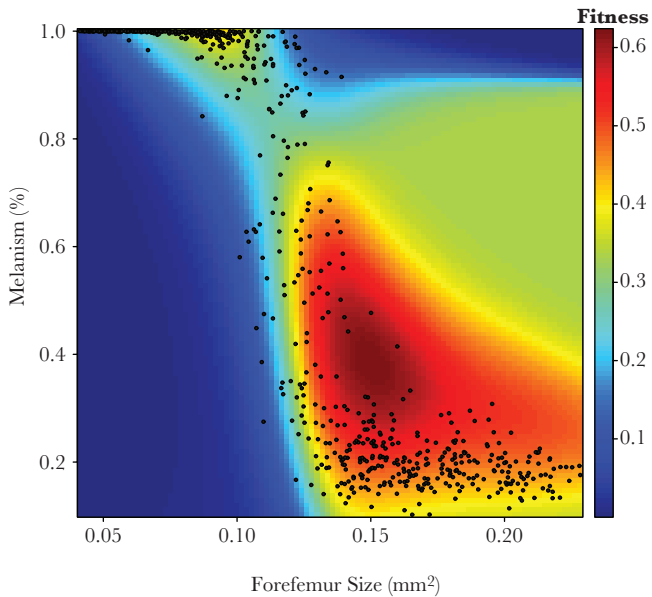


Figure 4
Two-dimensional fitness contour plot (forefemur size and melanism) for the *S. thoracica* males in the sexual selection experiment. The black dots represent the actual individual phenotypes.

body size and coloration at present also likely reinforces the coupling of the 2 traits, which may even be genetically linked via gene regulation (though we don't know), as they have been shown to be functionally linked, via the enzyme phenoloxidase, by a trade-off between the black color of the fly and its immune defense (Figure 1; Busso et al. 2017). However, whether this link is cause or consequence of sexual selection of course cannot be determined retrospectively at this point in time. We can only speculate that the strength of selection on coloration must have been relatively stronger than selection on body size at some earlier point in the evolution of this polyphenism, which was likely linked with the secondary evolution of male-biased sexual size dimorphism in this particular sepsid species (Rohner et al. 2016).

Accompanying disruptive sexual selection, we found 2 peaks in the males' fitness landscape (Figure 4). Strong phenotypic plasticity allows *S. thoracica* males to flexibly encompass both peaks with the same genotype. Polyphenism thus allows the species to avoid being trapped in either local optimum of the fitness landscape (Woodcock and Higgs 1996). Ambers occupy a higher fitness peak than obsidians, resulting in overall positive directional sexual selection for larger body sizes (Figure 4). This combination of disruptive and directional selection in *S. thoracica* could mediate the eventual evolutionary (i.e., genetic) escape from the lower (obsidians) to the higher optimum (ambers). Phenotypic plasticity will delay this escape until particular combinations of genetic changes appear (Phillips 1996; Weissman et al. 2009), which in sepsids are likely to arise eventually due to their large population sizes (Pont and Meier 2002). In general, plasticity therefore can facilitate the course of evolution by allowing a species to first explore the entire fitness landscape to then ultimately shift to a higher fitness peak through a combination of disruptive and linear selection.

The quasi dichotomous male coloration in *S. thoracica* could have also evolved as a status badge to signal the body size and fighting ability of an individual (Rohwer 1982). The honesty of the amber badge could have been maintained by the costs it incurs in terms of energy, physical injury and/or death (Geist 1974; Georgiev et al. 2013). Here, the amber coloration likely results in reduced immunocompetence of the individual (Busso et al. 2017). This cost would favor a sigmoid relationship with a trait threshold below which exhibiting the amber color would be detrimental in a highly competitive environment (Rohwer 1982), as only large enough individuals should afford the amber badge, ultimately producing a (genetic) linkage between body size and melanism. If a signal is an accurate predictor of the resource holding potential of an individual, such threshold traits can be adaptive (Svendsen et al. 2011). The bimodal fitness landscape in male *S. thoracica* encompassing a trade-off between body size and melanism supports the idea that disruptive selection favors threshold traits (Svendsen et al. 2011). The fitness trade-off between the mating advantage of large amber males and the better immune system of the small, melanic obsidians (Busso et al. 2017) thus can contribute to the discontinuous fitness optimum resulting in a bimodal fitness landscape (Chevin and Lande 2013), ultimately maintaining, if not producing, the 2 male morphs in *S. thoracica*.

Accompanying the morphological differences between morphs, we also found divergence in their behavior. In both experiments, ambers fought significantly more than obsidians, and the higher fighting frequency increased their mating success (Figure 3). Such condition-dependent behavior is likely integral part of the polyphenic syndrome. The benefit of aggression applies whether males fight directly for females, or whether they fight for territories on

dung pats granting them access to females coming to feed or lay eggs on the dung, both of which occur in this species (personal field observations). Regardless of the precise mechanism, our results concur with the typically greater mating success of aggressive individuals found in many other species (Dow and von Schilcher 1975; Gerlai et al. 2000; Brown et al. 2007; Spence et al. 2008).

Nevertheless, aggression also incurs costs in terms of energy, physical injury, and ultimately death (Geist 1974; Georgiev et al. 2013). Hence, individuals alternatively might employ signals to limit such fighting costs (Tinbergen 1951; Parker 1974; Rohwer 1982). We found that ambers waved more than obsidians in both experiments, which seems to result in less fighting (and waving) of obsidians with ambers at least in the sexual selection experiment (Figure 2a,c). However, waving did not significantly influence male mating success. This lack of effect could be either because waving is not an honest signal of an individual's resource holding potential, or because it has yet another meaning. Alternatively, female presence could have influenced male waving behavior in form of an audience effect, which can alter the original message of the signal (Zuberbühler 2008). Signals also typically depend on the environmental setting (Endler 1992; Schluter and Price 1993). As results from our 2 differing laboratory settings were not completely congruent, further experiments are needed to understand the signaling behavior of sepsid flies (cf. Puniamoorthy et al. 2009).

Regardless, there needs to be a mechanism regulating the frequency of the 2 morphs so that both can coexist in the long term. A threshold trait conditioned on body size seems to be an adequate solution to flexibly regulate the frequency dependent expression of behavioral tactics, as juvenile population density is typically negatively correlated with body size (Busso et al. 2017). At low densities the species can afford to produce only aggressive amber males ("hawks"; cf. Parker 1974) because the risks of fighting will be lower. As population density increases, fighting risks also increase and only the most competitive individuals will be able to bear the amber badge, which deters fights with obsidians (Figure 2). At very high densities the majority, if not all, individuals will be less aggressive obsidians ("doves"). Hence a threshold can provide a mechanism to balance the ratio between the 2 morphs and render optimal combinations of ambers (hawks) and obsidians (doves) according to the population density to minimize the fitness costs of each tactic. Note that this plastic regulation of morph frequencies under disruptive selection is an alternative evolutionary mechanism to classic frequency-dependent selection on genetically fixed genotypes, which we did not investigate here because in *S. thoracica* morphs are not fixed but largely plastic (Busso and Blanckenhorn 2018a). So, we cannot definitely exclude a role of negative frequency dependent selection in this system.

In contrast to male competitive behavior, female preferences did not play much of a role in our study, based on the laboratory settings and behaviors we assessed here. We found no specific female preferences for any morph, coloration or body size in the sexual selection experiment. This lack of preference was observed for both female mating behaviors studied (female reluctance to copulate and copulation latency). Hence, our combined evidence suggests that male-male competition is the primary diversification agent in this species (Seehausen and Schluter 2004), contributing to the male-biased sexual size dimorphism exhibited by the amber morphs (Rohner et al. 2016). However, one-on-one trials lacking any male-male interactions, which albeit unnatural arguably would be the best test of female preferences, were not performed here, so our conclusions about lacking female choice must remain limited (cf. Puniamoorthy 2014).

In our study, we estimated fitness benefits based on individual mating success, but as mentioned the amber morph may suffer other costs beyond mating, such as compromised immunity (Busso et al. 2017), energy loss, or physical injury (Geist 1974; Georgiev et al. 2013), all reducing survival. Additionally, body size affects fitness by influencing a large set of interrelated life-history traits (Blanckenhorn 2000). Further studies encompassing other fitness components are therefore the logical next step towards understanding the possible role of sexual selection for maintaining the male polyphenism in *S. thoracica*. For instance, predation could certainly additionally affect the costs and benefits of the morphs (cf. Busso and Blanckenhorn 2018c).

To conclude, we found evidence for intraspecific directional and disruptive sexual selection on male body size in the dimorphic fly *S. thoracica* that is mediated by male–male interactions, as also seen in dung beetles (Moczek and Emlen 1999, 2000), which contributes to the maintenance, if not the origin, of the male morphs in this species, together with other processes. Body size selection was strongly correlated with selection on coloration (melanism), in accordance with the functional link between the 2 traits (Figure 1), again presumably reinforcing that link. The morphological and quantitative behavioral differences between the morphs suggest the beginning of alternative reproductive tactics in this species as part of the entire condition dependent syndrome. Our study adds an important case in Diptera to an otherwise short list of examples of intraspecific disruptive sexual selection (Sappington and Taylor 1990; Greene et al. 2000; Stelkens et al. 2008; Busso and Davis Rabosky 2016). More generally, the simultaneous occurrence of disruptive selection and polyphenism in *S. thoracica* supports the role of sexual selection in the intraspecific diversification of various traits (Andersson 1994; Eberhard 2010), and ultimately could be a speciation starting point (Wittkopp et al. 2009; Corl et al. 2010). Disruptive selection acting within a species here supports the prospect of micro-evolutionary processes leading to reproductive isolation and ultimately sympatric speciation by sexual selection, a controversial issue in evolutionary biology (van Doorn et al. 2004; Gavrillets and Hayashi 2005).

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Busso and Blanckenhorn 2018b.

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